Observations on the Occurrence of the Prothallia of *Lycopodium inundatum*

JAMES G. BRUCE*,[1]

In the study of pteridophytes, the discovery of sexual plants is generally unusual. This is due in part to the small size of the gametophytes and in part to their commonly concealed habit. In the Lycopodiaceae alone, only 10% of the nearly 500 species have known gametophytes (Bierhorst, 1971). Discovering prothallia of pteridophytes under natural conditions is one of the most challenging problems of field botany.

In this paper I wish to report the first record of *Lycopodium inundatum* L. gametophytes for North America and to discuss their characteristics and the circumstances of their discovery.

The gametophytes of the Lycopodiaceae have interested botanists for almost a hundred years since the first discovery of naturally occurring gametophytes (Fankhouser, 1873). Our understanding of these plants, however, has been greatly hindered by the rarity of collections. The most important contributions to our knowledge were all carried out well over 50 years ago by only a few notable workers. They established the taxonomic distribution of the types of gametophytes and also the major stages in the development of the gametophyte and embryo.

Treub (1884; 1886a,b; 1888a,b; 1890a,b,c) in Java described the gametophyte and embryo of *L. cernuum* L. and several other tropical species. Goebel (1887) found and described the gametophyte of *L. inundatum* L. in Europe. This is the only time the gametophyte of this species has ever been reported, as far as I have been able to determine. Probably the best known work on the prothalli of the Lycopodiaceae is that of Bruchmann, who essentially laid the foundations for the systematic study of these plants. It was he who first described (1885, 1898, 1908, 1910) in detail the gametophytes of the temperate species *L. annotinum* L., *L. clavatum* L., *L. complanatum* L. (which may actually have been *L. tristachyum* Pursh according to Wilce, 1965), and *L. selago* L. Bruchmann also succeeded in growing the prothalli of *L. annotinum, L. clavatum,* and *L. selago* from spores, which required in some cases 6–7 years to germinate and 12–15 years to mature. An excellent review of the early attempts to germinate the spores of *Lycopodium* species was made by Chamberlain (1917). Freeberg and Wetmore (1958), in their study of the culture of *Lycopodium* gametophytes *in vitro,* have summarized in greater detail than is done here the contributions of Bruchmann, Treub, and Goebel.

Bruchmann (1898) divided the gametophytes of *Lycopodium* into five categories based on habit and anatomical organization. Three of these types are sub-

* Department of Botany, University of Michigan, Ann Arbor, MI 48104.

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terranean and characteristic of most terrestrial members of the genus. Another is epiphytic in bark humus and is characteristic of many tropical species. The last is green, superficial on the soil, and is typical of the bog clubmosses. The third type is also found in the gametophyte of *Phylloglossum,* a closely related genus.

Gametophytes of the green, superficial type have been illustrated for *L. alopecuroides* L. (Eames, 1942), *L. cernuum* L. (Treub, 1884), *L. inundatum* L. (Goebel, 1887), *L. laterale* R. Brown (Chamberlain, 1917), *L. ramulosum* Kirk (Holloway, 1920), and *L. salakense* Treub (Treub, 1888a).

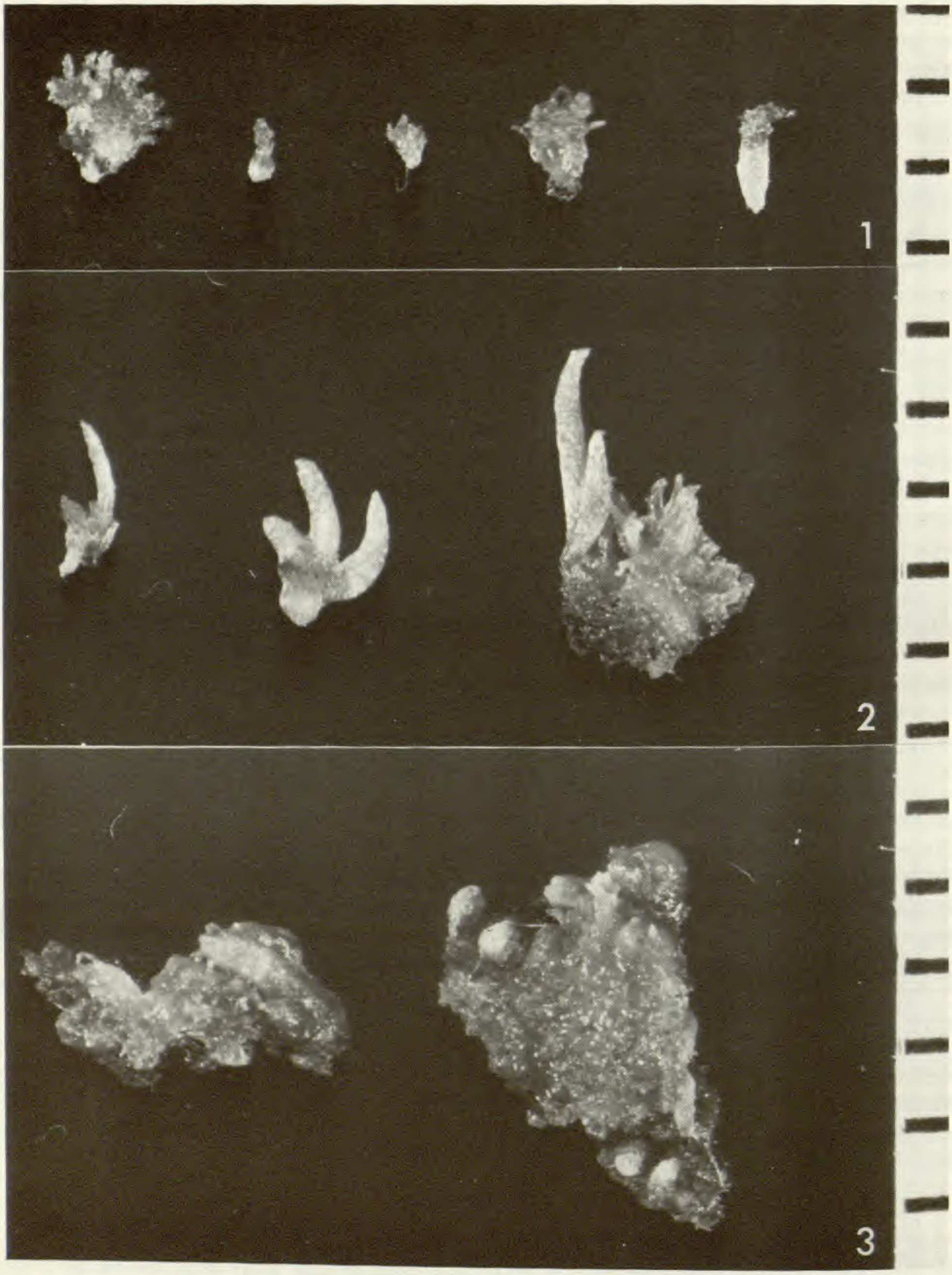
I sought gametophytic material for purposes of confirming earlier reports, as well as to enable additional studies involving growth and development of the gametophyte and the embryogeny of the young sporophyte. *Lycopodium inundatum* is the most abundant of the bog clubmosses in Michigan. It occurs in numerous localities throughout much of the state. The most productive of these have been the many borrow pits which flank new highways and are the source of fill dirt. These often form sandy, damp depressions which are ideal for the growth of these plants. Gametophytes of *L. inundatum* have been found in two localities, one in Midland County in the central part of the lower peninsula of Michigan and the other some 130 miles distant in Van Buren County in the southwestern part of the lower peninsula. Mature sporophytes were present in large numbers and were seen growing approximately one meter from the gametophytes in both localities.[2] However, in the Midland County site large colonies of sporophytes were no nearer than 10 meters. Both sites were visited in the late summer and early fall. In addition, the Midland County site was visited in the early spring.

The following plants were noted as associates in the Midland County locality: *Equisetum arvense, Gerardia tenuifolia, Lycopus uniflorus, Salix rigida, Salix alba* × *fragilis, Solidago graminifolia, Typha angustifolia,* and *Viola lanceolata.* The Cat-tail was the most conspicuous element in the immediate area. The gametophytes were confined to the areas between the tufts of Cat-tail. These surfaces were typically open with little other vegetation present. The microhabitat presented a minutely rough terrain. Numerous tiny hill-and-valley situations were present with the gametophytes more plentiful in the valleys and on the slopes than on the more exposed crests.

At the Van Buren County site, the location of the gametophytes within the microhabitat was essentially the same as at the Midland County locality. Here, however, the gametophytes were confined to a raised, sandy region along a small stream. Mature sporophytes were present along both banks of the stream, but not on the sand bar where the gametophytes were found. The sand bar was covered by a more or less open growth of grasses and sedges with bare ground between the tufts. The sandy soil was moist probably because of the proximity of the stream.

Locating the gametophytes is a difficult process. Habitats were first visited where mature colonies of sporophytes were known. Within these, likely areas were selected

[2] In the Van Buren County locality some of the gametophytes found may belong to a closely related variety or species, the sporophyte of which has been detected at this locality, growing with typical *L. inundatum,* and which will be reported in detail in the near future.



Lycopodium inundatum. Fig. 1. Young gametophytes. Fig. 2. Gametophytes with young sporophytes attached. Fig. 3. Massive gametophytes with several attached sporophytes. Scale in millimeters.

which possessed numerous, small (up to 1 cm long) sporophytes. If closer examination of these areas with a hand-lens revealed any gametophytes, soil turfs approximately 20 cm² were removed and transported to the laboratory where they were carefully examined with a dissecting microscope. Utilizing this technique, I discovered over 100 gametophytes, many of them with attached young sporophytes, from the Midland County site, and about 25 from the Van Buren County site.

Within the collections there is considerable variation in the size of individual gametophytes, with no break in the range of sizes. Some are quite massive and bear as many as five young sporophytes; others are much smaller and appear to lack sex organs.

There was a great variety of ecological situations in the microhabitats with respect to light, moisture, topographic, and edaphic factors. Spores doubtless fall into most of these different ecological sites. As some of these are more suitable for germination than others, variation with respect to time of spore germination is likely. Similarly, gametophyte growth may be expected to vary according to micro-environmental factors. Both factors give rise to size variation in individual plants.

The Midland County site was visited in early April to determine whether gametophytes survived winter conditions. Previous reports on this point were unclear; the undefined term "short-lived" frequently has been applied to the gametophytes. The following observations show that some prothallia survive winter in a mature state and suggest that survival for more than one season is possible. The locality at the time of our visit was covered with about 5 cm of ice; several centimeters of snow covered most of the area. Up to 8 cm of water was observed under the ice. The ground was not frozen, and soil turfs were removed easily. The only real difficulty was choosing the correct point to cut through the ice. Soil turfs taken from the site revealed several gametophytes of various sizes. The largest of these was 2.2 mm long by 0.7 mm diameter and is quite comparable to those of *Fig. 1*. The gametophytes appeared to be entirely healthy judging from turgidity and green color.

An additional factor affecting gametophyte growth is the endophytic fungus. Freeberg (1962), in his study of the endophyte-prothallus relationship *in vitro*, points out that on starch media he was able to obtain greater prothallial growth with an endophytic fungus present than in those prothallia cultured in its absence. He also states that the fungus assists in this growth only when it has actually entered the prothallus. In earlier work, de Bary (1858) germinated the spores of *L. inundatum* and grew them to a maximum of 11 cells. They would grow no larger in culture. Treub (1884) germinated the spores of *L. cernuum* and grew gametophytes up to 10 cells; he asserted that invasion by the endophytic fungus was necessary to insure further development.

It thus seems probable that a combination of factors such as variation in the time of germination of spores, variation in the growth rate of individual gametophytes, variation in the time of encounter with an appropriate endophyte, and overwintering could all lead to variation in the size of individual gametophytes collected at any one time.

The gametophytes themselves are relatively inconspicuous; the largest rarely exceed 5 mm in diameter. Usually the gametophyte resembles a small, upright cylinder partially embedded in the soil with a somewhat expanded aerial portion (*Fig. 1*). The distal expansion is covered by few to many planate lobes one to several cells thick, which impart a bushy appearance to the larger specimens. The sex organs are situated near or at the bases of these lobes. The lower region of the gametophytes is invested with numerous, non-septate rhizoids. The endophytic fungus is confined to this basal region in healthy gametophytes. In necrosing prothallia the fungus assumes a much greater dimension in the affected regions and may eventually completely overrun the gametophyte.

Fig. 4. Longitudinal section of the archegonium of *Lycopodium inundatum* through its axis; n = neck cell.

Young sporophytes are attached peripherally to the prothallus; the size of prothallia with developing sporophytes varies tremendously (*Figs. 2* and *3*). Gametophytes with attached sporophytes appeared progressively more necrotic with increasing size of the sporophyte. Gametophytes without visible, attached sporophytes almost invariably appeared healthy. This suggests that one factor leading to gametophyte death may simply be fertilization and consequent embryo development.

In addition, gametophytes were sectioned by paraffin technique and photomicrographs were made. In the development of the archegonia of *L. inundatum,* relatively few tiers of neck cells are produced. The specimens seen so far show only three tiers, but this may not be the maximum. However, all but the inner tier are sloughed off when, or soon after, the egg matures. Numerous archegonia

with presumably unfertilized eggs possess only a single tier of neck cells (*Fig. 4*). Old archegonia associated with young embryos often show the single tier of neck cells if embryo development has not yet obliterated the essential features. These neck cells appear to have slightly thickened walls, compared with adjacent cells. A similar situation apparently occurs in *L. cernuum,* judging from the illustrations of Treub (1884). Both of these situations appear to be analogous to at least one of the conditions that is seen in the development of archegonia in *Psilotum* (Bierhorst, 1954).

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